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# The Effect of Nrf2 on Diabetic Complications

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## Abstract

The Nrf2 has been identified as a key molecular player in orchestrating adaptive cellular interactions following a wide spectrum of cellular conditions that could be either extracellular or intracellular. The encoded transcription factor regulates genes, which contain antioxidant response elements (ARE) in their promoters; many of these genes encode proteins involved in response to environmental stress, detoxifying enzymes, metabolic enzymes, injury, and inflammation, which includes the production of free radicals. The association between oxidative stress and inflammation with progression of diabetic nephropathy and cardiomyopathy has been described. The prevention of diabetic nephropathy and cardiomyopathy has become a global concern for those who are working in diabetic care management. Therefore, activation of Nrf2 has the potential to protect against macromolecular damage. Studies have demonstrated the beneficial role of Nrf2 induction in the prevention of DN. Upon exposure of cells to oxidative stress or electrophilic compounds, Nrf2 dissociates from Keap1 and translocates into the nucleus to bind to antioxidant-responsive elements in the genes encoding antioxidant enzymes. Upregulation of these Nrf2-dependent antioxidants promotes detoxification and anti-inflammatory function. Thus, the Nrf2 activators have been suggested for preventing diabetic nephropathy.

**Keywords:** Nrf2, diabetic complications, nephropathy diabetic

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## 1. Introduction

The stimatives demonstrated that 10% of the global population will have diabetes by 2035 [1]. Hyperglycemia, hyperlipidemia, and inflammation are the metabolic abnormalities in diabetes and are involved with the development of reactive oxygen or nitrogen species (ROS or RNS) [2].

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Diabetes is a strikingly heterogeneous disease with variable clinical, pathologic, and molecular features [1]. It has been described that high glucose induces the oxidative damage and it is related potentially on the diabetic complications in animal models [3].

Additionally, it is known that Nrf2 expression increases in response to oxidative stress [2]. The Nrf2 transcription factor (nuclear factor, erythroid 2-derived 2-like 2 or nuclear factor erythroid 2-related factor 2) has been identified as a key molecular player in orchestrating adaptive cellular interactions following a wide spectrum of cellular conditions that could be either extracellular or intracellular [3].

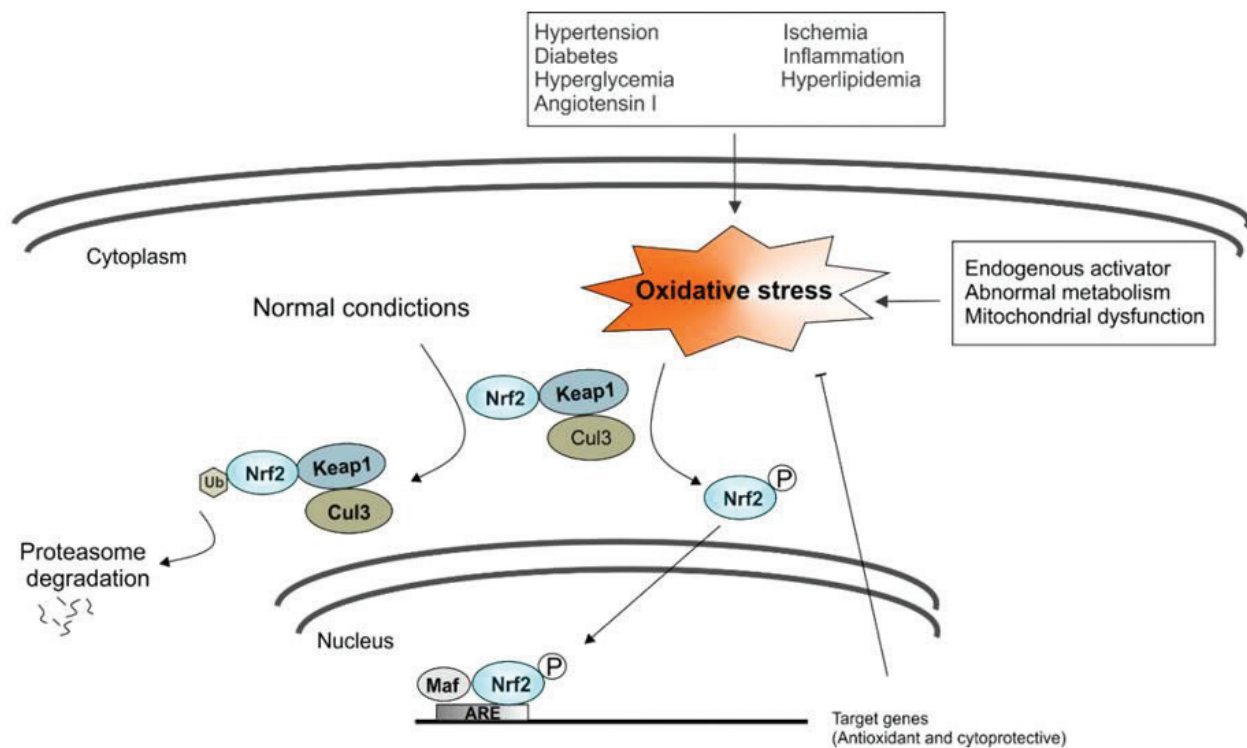
Upregulation of Nrf2 and/or its downstream antioxidant genes in response to hyperglycemia is of growing interest in the clinical and research community [2]. The Nrf2 is a master regulator of cellular detoxification response and redox status and also provides a protective action from various oxidative stresses and damages [1, 2]. Given that we have yet to understand molecular mechanisms of diabetes complications, the results of studies may play an important role in elucidating the molecular pathways and get the development of strategies for prevention, treatment, and management of macrovascular and microvascular diseases (diabetes complications).

## 2. Implications of Nrf2 transcription factor in diabetic complications

The gene *Nrf2* also known as *NFE2L2* encodes a transcription factor that regulates genes that contain antioxidant response elements (ARE) in their promoters; these genes encode proteins involved in response to environmental stress, detoxifying enzymes, metabolic enzymes, injury, and inflammation, which includes the production of free radicals. During nonstressed conditions, the Nrf2 is inactive in the cytoplasm connected to protein Kelch-like ECH-associated protein 1 (Keap1), which prevents its translocation to the nucleus [4].

During oxidative stress, a signal that involves phosphorylation and/or redox modification is transduced to the Keap1/Nrf2 complex (see **Figure 1**), leading to its disruption and nuclear translocation of Nrf2. However, under basal conditions, Keap1 mediates rapid ubiquitination and subsequent degradation of Nrf2 by the 26S proteasome [3, 4]. Cullin 3-based ubiquitin E3 (Cul-E3) ligase complex ubiquitinates Nrf2, and Keap1 serves as a substrate adaptor, which facilitates the ubiquitination of Nrf2 by Cullin 3. As a result, Nrf2 has a short half-life that lasts only 20 min under normal conditions. Oxidative stress destroys critical cysteine residues in Keap1, disrupting the Keap1-Cul3 ubiquitination system. If Nrf2 is not ubiquitinated, it builds up in the cytoplasm and is translocated into the nucleus [4–6].

In the nucleus, Nrf2 combines with a small protein called Maf to form a heterodimer, and, by binding to the ARE in the upstream promoter region, it initiates the transcription of various cytoprotective genes including those encoding antioxidant and phase II detoxifying enzymes such as catalase (CAT), superoxide dismutases (SODs), heme oxygenase-1 (HO-1), NAD(P)H dehydrogenase (quinone) 1 (NQO-1), glutathione peroxidase-1 (GPx-1), glutathione S-transferase (GST), and  $\gamma$ -glutamylcysteine synthase ( $\gamma$ -GCS). The antioxidant response provided by the NFE2L2 and Keap1-NFE2L2/ARE signaling pathways protects the pulmonary, hepatic, digestive, neural and cardiovascular systems, and Nrf2 is considered a



**Figure 1.** Schematic presentation of Nrf2 pathway activation by reactive oxygen species (ROS). Under basal conditions, Keap1 mediates rapid ubiquitination and subsequent degradation of Nrf2 by the 26S proteasome. During oxidative stress, the Nrf2 regulates genes, which contain antioxidant response elements (ARE) in their promoters.

promising target against diabetic complications such as cardiovascular diseases and diabetic nephropathy [7–9].

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, with or without defects in insulin production and secretion, and as the prevalence of obesity in children has increased, T2DM has also become more common. T2DM is characterized by insulin resistance (with or without defects in insulin production and secretion), and the routine medical treatment is challenging, especially the identification and management of complications associated with micro- and macrovascular damage in diabetes [10].

Diabetes is a multifactorial process involving genetics, lifestyle, ethnic and racial heritage, and environmental factors. On the other hand, the interplay of these factors is not yet understood. The single nucleotide polymorphisms (SNPs) are a common type of genetic variations, which have been shown to impact most population susceptibility to diseases and individual response to drug treatments [10, 11].

In Chinese population was investigated NFE2L2 SNPs for possible associations with either T2DM or diabetic complications such as diabetic foot, microangiopathy and peripheral neuropathy, nephropathy, and retinopathy. This study showed that the T2DM patients with complications presented a higher frequency of mutant allele than the T2DM patients without complications. The study suggests that NFE2L2 SNPs are associated with T2DM patients with complications [10].

With oxidative stress and chronic systemic inflammation inseparably interconnected, inhibiting oxidative stress was theoretically an effective strategy to delay diabetes-related macrovascular and renal diseases. Research has revealed that the expressions of Nrf2-mediated anti-oxidative enzymes, including HO-1, NQO-1, and GPx-1, were significantly increased in the diabetic kidney when treated with salvianolic acid A (SAA) alone or in combination with metformin (MET). SAA is a polyphenol derivative extracted from the root of *Salvia miltiorrhiza*, which is known to show a variety of pharmacological activities including antioxidant, anti-inflammatory, and antiplatelet properties [8, 9].

Furthermore, SAA alleviates H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress via activation of Nrf2/HO-1 signaling. The MET is an oral hypoglycemic agent, which is widely used in patients with type 2 diabetes. MET decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization [8–10].

Compared to treatment with either SAA or MET alone, their combination provided further protection against the macrovascular and renal injury, which was at least partly due to therapeutic activation of both MET-mediated AMP-activated protein kinase (AMPK) and SAA-mediated Nrf2/antioxidant response elements (ARE) pathways [12]. It is suggested that polyphenol Nrf2 modulators, especially combined with drugs activating AMPK including hypoglycemic drugs, are worthy of further investigation to combat diabetic complications [9].

The genetic deficiency in Nrf2-mediated transcriptional responses, especially antioxidant pathways, enhances susceptibility to both ischemic and nephrotoxic AKI in mice. Nrf2 deficiency is associated with an increased mortality accompanied by augmented kidney dysfunction and vascular permeability. Liu et al. [13] demonstrated that Nrf2 deficiency enhances susceptibility to both ischemic and nephrotoxic acute kidney injury and identifies this transcription factor as a potential therapeutic target in these injuries.

On the other hand, Nrf2 plays a protective role in experimental acute kidney injury, and this protection is mediated, in part, through an endogenous antioxidant pathway [2, 13].

Diabetic cardiomyopathy (DCM) is one of the major cardiac complications in diabetic patients. DCM is related to oxidative stress that is due to imbalance between ROS and/or reactive nitrogen species generation and their clearance by antioxidant defense systems [2–6]. Experimental and clinical studies have shown the important roles that Nrf2 and its downstream genes play in the pathogenesis of cardiac remodeling and heart failure induced by a number of factors. TNF- $\alpha$  has an important role in a number of pathologies associated with oxidative stress including diabetes, cancer, cardiac hypertrophy, and cardiomyopathy [6, 13].

The exposure of TNF- $\alpha$  to cells at concentrations well below the threshold associated with subinflammation significantly increased Nrf2 activity and its nuclear translocation. The TNFR1/2 double knockout mice and HL-1 cardiomyocytes make a first step forward in understanding the bimodal effects of the cytokine, TNF- $\alpha$ , in regulating the redox-sensitive Keap1/Nrf2 antioxidant pathway. This study has potential importance in the field of cardiovascular signaling because the TNF- $\alpha$ -induced biphasic regulation of the Nrf2 pathway suggests that



a certain threshold of TNF/ROS signaling is essential to prime and activate the Nrf2 protective signaling pathway [14].

Research revealed that broccoli sprout extract (BSE), a natural SFN-rich supplement, can prevent the development of DCM. Like sulforaphane (SFN, an Nrf2 activator), BSE can prevent DCM in a transgenic T2DM mouse model via activation of Nrf2 by inhibiting diabetes-induced cardiac oxidative stress and damage as well as inflammation. BSE, when used at higher doses, can be used as a natural and safe source of SFN to upregulate Nrf2 expression and prevent DCM. Similar to its reported beneficial impact when used in patients with other chronic diseases, our results demonstrate that BSE also has promising potential in the treatment of patients with diabetes mellitus. Treatment with SFN-rich BSE for 3 months could significantly prevent the pathological process of DCM in the T2DM mice model, and like SFN, BSE also significantly upregulated Nrf2 expression and function to prevent diabetes-induced cardiac oxidative stress and inflammation. Therefore, BSE could potentially be used as a natural and safe treatment against DCM via Nrf2 activation [11].

Coronary artery disease and ischemic heart disease are prevalent worldwide. The development of percutaneous coronary intervention and surgical revascularization has brought marked benefits to patients with acute MI. However, ischemia/reperfusion injury during revascularization can cause further cardiac injury. Nrf2 and its target genes have been shown to play a protective role in cardiac ischemia-associated injury. Some antioxidants protect the heart from ischemia-induced cardiac injury via the Nrf2 pathway. For example,  $\alpha$ -lipoic acid and prostaglandin D2 significantly increased Nrf2 nuclear translocation; the expression of its downstream genes reduced lactate dehydrogenase (LDH) and creatine kinase (CK) release, attenuated myocardial infarct size, decreased cardiomyocyte apoptosis, and partially preserved heart function; and this effect was at least partially PI3K/Akt signaling pathway dependent [6, 14, 15].

In adipose tissues, ROS promote the conversion from preadipocytes to mature adipocytes and facilitate insulin action [16, 17]. The ROS-mediated biological signaling pathways could be affected by enhanced Nrf2-ARE activity because ROS signaling intermediates should inversely correlate with the ROS scavenging activity and antioxidant status in cells. Thus, when the cells are chronically exposed to oxidative stressors, cellular ROS scavenging capacity is adaptively upregulated, primarily through the activation of Nrf2 and subsequent transcriptional induction of a suite of antioxidant enzymes [11].

Additionally, the induced antioxidant enzymes may have the undesired effect of impeding the physiological role of ROS as signaling molecules. Although antioxidants protect adipocytes from oxidative damage, they also may blunt aspects of ROS signaling, resulting in reduced adipogenesis and insulin resistance. Nrf2 controls white adipose tissue expandability and serves to maintain glucose and lipid homeostasis, including control of adipogenesis [19–21]. In addition, ROS, whose cellular concentrations decline with increases in Nrf2-regulated antioxidant gene expression, also affect insulin signaling [17, 18].

It is described that oxidative stress is a dynamic condition characterized by an imbalance between pro-oxidants and antioxidants. Reactive oxygen species (ROS), not adequately

counterbalanced by antioxidant defenses, causes DNA damage. However, it is the action of antioxidants such as the Nrf2 transcription factor that acts by activating cytoprotective genes, which promote cell survival. In basal conditions, the repressor protein Keap1 binds Nrf2 in the cytoplasm and promotes its degradation. In the presence of ROS, Keap1 is inactivated and releases Nrf2 resulting in its nuclear translocation. The presence of mutations of the Nrf2-Keap1 genes and favoring greater Nrf2 expression and action have been described in association with different types of diseases. Recently, we have demonstrated the critical role of Nrf2 expression in protecting the cardiac cells from oxidative damage and death caused by high levels of glucose [19, 20, 22]. Thus, the cells have evolved endogenous defense mechanisms against sustained oxidative stress.

Experimental studies have shown that oxidative stress directly induces insulin resistance in cardiomyocytes via exaggerating extracellular signal-related kinase (ERK) activity in vitro. Additionally, depressed expression of cardiac Nrf2 was associated with significant increases in nitrosative damage and phosphorylation of ERK, all of which were prevented in the hearts of diabetic mice with cardiac overexpression of a potent antioxidant metallothionein (MT) [23, 24].

On the other hand, upregulation of cardiac Nrf2 by its activator dihydro-CDDO-trifluoroethyl amide (Dh404) significantly prevented diabetes-induced nitrosative damage, ERK activation, and insulin signaling downregulation. Thus, these findings suggest that oxidative stress–depressed expression of cardiac Nrf2 is associated with cardiac activation ERK and downregulation of glucose metabolism [25]. The studies suggested that the Nrf2 is a master transcriptional factor of antioxidative defense system [19] and may be a novel negative regulator of oxidative stress–mediated insulin resistance in cardiomyocytes and the heart [22].

Traditionally, interactions among metabolic and hemodynamic factors are considered to be involved in the development of renal lesions in patients with diabetes, for example, diabetic nephropathy (DN). However, several other factors such as oxidative stress and inflammatory processes have been shown to play important roles in the pathogenesis of DN; these factors are not completely independent, but they interact with each other. Several studies report the infiltration of macrophages and proinflammatory cells in kidney at different stages of DN [26]. The inflammatory infiltrate produces reactive oxygen species (ROS), proinflammatory cytokines, and growth factors, which lead to upregulation of chronic systemic inflammation and mediate the progression of diabetic nephropathy [26, 28–30].

As a consequence of inflammation, a variety of cytokines and acute phase proteins are released in order to augment or attenuate the inflammatory response. The main inflammatory cytokines involved in the development of DN are interleukin 1 (IL-1), IL-6, and IL-18 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ); these might contribute to the progression of renal injury either directly or indirectly [27]. Thus, chronic inflammation contributes to DN not only as a consequence of a direct effect of proinflammatory mediators on cellular signaling but also by creating a state of oxidative stress. In recent years, several investigators have provided substantial evidence implicating nuclear factor Nrf2 in inflammation and associated disorders [30, 31].

The therapeutic potential of Nrf2 activation in diabetes, implicating control of oxidative stress, in addition to regulation of inflammatory cytokines as methods of Nrf2 protection, was described. Also, it has been described that severe oxidative stress is associated with inflammation in chronic kidney disease (CKD) [27]. Oxidative stress and inflammation are mediators in the development and progression of chronic kidney disease (CKD) and its complications, and they are inseparably linked as each begets and amplifies the other. Although pathways involved in intrarenal ROS production and inflammation in experimental CKD have been widely explored, the relationship of DN on proinflammatory cytokines and Nrf2-Keap1 system in diabetes is poorly studied [27, 28, 30].

There was no an effective approach to prevent the development of these complications for the patients with diabetes. Thus, diabetic nephropathy and cardiomyopathy are the two major causes for the mortality of diabetic patients. Although there remain many questions to be further investigated, the potential beneficial effects of upregulation of Nrf2 and/or its downstream protective components have attracted the attention of basic researchers and clinical physicians to consider its potential application in the clinic [5–30].

### **3. Strategies to prevent diabetic complications by activating Nrf2 factor**

The glucose control, blood pressure, lipid lowering, and the blockage of the renin-angiotensin systems were used for the treatment of diabetic patients, and the development and progression of nephropathy and cardiomyopathy in the patients with diabetes remain unpreventable [2]. Ultimately, it can be inferred that these Nrf2 cross talks with other signaling pathways are of clinical importance within the context of many human disease condition pathogenesis, particularly those with highly complex multifactorial molecular interaction, as in diabetic complications [32].

Diabetic nephropathy (DN), one of the major microangiopathic chronic diabetic complications, is associated with an increased risk of major cardiovascular events and all-cause mortality. DN is now the major cause of chronic kidney disease throughout the world and is the largest single cause of end-stage renal disease, accounting for nearly half of the patients entering dialysis each year. The etiopathogenesis of DN is clearly multifactorial, including genetic and environmental factors. Evidence indicates that mechanisms are active by mitochondrial overproduction of reactive oxygen species (ROS) [34, 37].

Extra generation of ROS, induced by hyperglycemia, is considered as the main reason for the development of these diabetic complications. Oxidative stress contributes to the pathogenesis of diabetic nephropathy. Nuclear factor erythroid-derived 2-like 2 (NRF2) controls cellular defense mechanisms against oxidative stress by turning on transcription of antioxidant genes [33]. Under physiological conditions Kelch-like ECH-associated protein 1 (Keap1) binds to Nrf2 and sequesters it in the cytoplasm. Under basal conditions, Keap1 mediates rapid ubiquitination and subsequent degradation of Nrf2 by the proteasome [7, 35, 36, 38]. Studies have demonstrated the beneficial role of Nrf2 induction in the prevention of DN [37].



Upon exposure of cells to oxidative stress or electrophilic compounds, Nrf2 dissociates from Keap1 and translocates into the nucleus to bind to antioxidant-responsive elements in the genes encoding antioxidant enzymes. Upregulation of these Nrf2-dependent antioxidants promotes detoxification and anti-inflammatory function. A growing body of evidence has indicated a critical role for activator-induced Nrf2 upregulation in the prevention of diabetic complications, including DN [36].

Other important targets of the Nrf2/Keap1/ARE system are lipogenic genes involved in regulation of triglyceride and cholesterol synthesis and metabolism, such as sterol response element-binding protein-1 and fatty acid synthase, where activation of Nrf2 leads to downregulation of lipogenic gene expression, affording protection against lipogenic stress. Repression of sterol response element-binding protein-1 decreased expression and activities of diacylglycerol acyltransferase-1 and 2 activity and fatty acid synthase, leading to decreased synthesis of triglycerides and cholesterol esters and secretion of apolipoprotein B100 in very low-density lipoprotein. Therefore, activation of Nrf2 has the potential to protect against macromolecular damage and against metabolic dysfunction and dyslipidemia [37].

Given these considerations, the preventive effect of Nrf2 activation on diabetic complication in animal models has been explored recently. The Nrf2 activators have been suggested for preventing diabetic nephropathy such as insulin, sulforaphane (SFN), cinnamic aldehyde (CA), and others. It is known that NQO1, one important Nrf2 downstream protective component, which is an important detoxifying enzyme that protects against diabetic complications. Functional variants of NQO1 were associated with the development of coronary artery disease in people with type 2 diabetes [35].

Studies have shown that after chronic treatment with SFN, that diabetic mice exhibited significant renal protection from diabetes-induced damage most likely via induction of Nrf2-mediated antioxidant pathway. SFN has garnered particular interest as an indirect antioxidant due to its extraordinary ability to induce expression of several enzymes via the Keap1/Nrf2 pathway. The studies indicate the requirement of Nrf2 for SFN and CA-induced renal protection against diabetes [37]. The activation of Nrf2 by SFN is able to suppress hyperglycemia-induced oxidative stress and metabolic dysfunction in human microvascular endothelial cell [37].

Anti-inflammatory, antioxidant, and antimicrobial effects of curcumin products have been widely investigated recently [9–41]. The analog C66 from curcumin was found to effectively inhibit high glucose (HG)-induced inflammatory response and macrophage infiltration, resulting in a significant prevention of renal injury in diabetic rats, response, and macrophage infiltration, resulting in a significant prevention of renal injury in diabetic rats. Liu et al. [39] demonstrated for the first time that C66 can protect the aorta from diabetes using a type 1 diabetes mouse model. Treatment of diabetic mice with C66 for 3 months can almost completely reverse and/or prevent the progression of diabetes-induced aortic oxidative damage, inflammation, apoptosis, and proliferation.

C66 is a novel curcumin analog with a much lower effective dose of 5 mg/kg administered every other day and has been shown to establish protection from diabetic nephropathy and diabetic cardiomyopathy [7, 40]. Wu et al. [8] have demonstrated that in addition to upreg-

ulating Nrf2 by increasing miR-200a, C66 also protects against DN by inhibiting miR-21. Curcumin is a regulator of epigenetic events, including miRNAs, and among miR-21 has been demonstrated to play a key role in the pathogenesis of DN. Liu et al. described that C66's renal protection from diabetes was accompanied by a significant inhibition of c-Jun N-terminal kinase (JNK). Inhibition of JNK phosphorylation by C66 and JNKi also significantly prevented diabetes-induced increase in inflammation, oxidative and nitrative stress, apoptosis, cell proliferation, and fibrosis [39].

The health-beneficial effects of fruit and vegetables have been linked to their content of activators of Nrf2. Key dietary bioactive activators of this system are glucosinolate-derived dietary isothiocyanates (SFN) and indoles, thioethers and disulfides, polyphenols, flavonoids, carotenoids, oxidized omega-3 fatty acids, and triterpenoids. Additionally, SFN is found in the brassica vegetables (broccoli, cabbage, cauliflower, Brussel sprouts, and others) and rocket salad [11]. The diet as a source of anti-inflammatory and health-beneficial compounds for patients with chronic renal failure has been recognized. With markedly decreased clearance in this patient group and adverse effects of some dietary bioactive compounds at high doses, particularly glucosinolate-derived isothiocyanates, it is necessary to proceed cautiously with dietary recommendations [38].

The activation is thought to occur by different mechanisms: SFN releases Nrf2 from Keap1 by modification of critical cysteine thiol residues; some polyphenols induce downregulation of Keap1 expression and/or induce mild oxidative/nitrosative stress; and J3-isoprostanes disrupt the Keap1-Cul3 complex, preventing Keap1-Nrf2 targeting to the proteasome. Many chemically diverse activators have already been identified, including the glutathione peroxidase-1, SN found in cruciferous vegetables, caffeic acid phenethyl ester from the bee product propolis, and CA (found in cinnamon bark) [37]. Many have shown promising actions relevant to diabetes complications [38]. Dietary habits may also be difficult to change, but processing of food products and beverages may also be modified to increase levels of Nrf2 activators in foodstuffs. It is generally accepted that a diet rich in fruits and vegetables helps stave off the development of cardiovascular disease [38].

Dietary and synthetic activators use as secondary prevention measure for DN should remain a top priority for health official campaigns [41]. To reach the public health goal of reducing DN prevalence, campaigns to engaging in diabetic complications prevention need to be addressed, including dietary education strategies.

## 4. Conclusions

Oxidative stress is a major player in the etiology of diabetic complications. Given these considerations, the Nrf2 factor is a master regulator of redox homeostasis and the cellular detoxification response. It has been demonstrated that natural compounds derived from plants, vegetables, and micronutrients can activate Nrf2 and, thus, promote antioxidant pathways to mitigate oxidative stress and hyperglycemic damage. Studies are needed to evaluate the potential effect of Nrf2 activators. Thus, these activators play an important role

in stress oxidative, such as a therapeutic strategy in preventing the development of diabetic complications.

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